*Br. J. Pharmac.* (1981), **73**, 681–689

# THE EFFECT OF ANAESTHETICS ON THE UPTAKE AND RELEASE OF γ-AMINOBUTYRATE AND D-ASPARTATE IN RAT BRAIN SLICES

M.C.W. MINCHIN[1]

Department of Physiology, University of Sheffield, Sheffield S10 2TN

**1** The effect of various concentrations of thiopentone, pentobarbitone, methohexitone, hydroxydione, alphaxalone/alphadolone, ketamine, α-chloralose and urethane on the transport of radiolabelled γ-aminobutyric acid (GABA) and D-aspartate was investigated.

**2** Uptake of the amino acids was weakly inhibited, if at all, by the anaesthetics and it is unlikely that such effects contribute significantly to their physiological function.

**3** The spontaneous efflux of GABA and D-aspartate was not detectably altered by any of the drugs tested.

**4** Thiopentone, pentobarbitone, methohexitone and hydroxydione inhibited $K^+$-stimulated GABA and D-aspartate release. The other anaesthetics had no effect on $K^+$-stimulated amino acid release.

**5** The rank order of potency of the inhibitors of $K^+$-stimulated amino acid release did not correlate with their anaesthetic potency. Furthermore not all inhibitors appeared to be very effective at anaesthetic concentrations.

**6** It is concluded that although it is possible that inhibition of excitatory transmitter release may be involved in the anaesthetic action of some anaesthetics, for many of the substances tested in this study such a mechanism does not appear to be implicated.

## Introduction

A considerable amount of evidence suggests that intravenous anaesthetics, and barbiturates in particular, interact with inhibitions thought to be mediated by γ-aminobutyric acid (GABA) in the mammalian central nervous system. This includes the prolongation and enhancement *in vivo* of postsynaptic inhibition on hippocampal pyramidal cells (Nicoll, Eccles, Oshima & Rubia, 1975), mitral cells of the olfactory bulb (Nicoll, 1972) and Purkinje cells of the cerebellum (Eccles, Faber & Táborikova, 1971) and of presynaptic inhibition in the spinal cord (Eccles, Schmidt & Willis, 1963). Such enhancements could result from (a) increased release of GABA, (b) decreased uptake of GABA, (c) increased efficacy of GABA at the postsynaptic membrane or any combination of these. There is good evidence for (c) at a variety of membrane sites including frog spinal neurones (Nicoll, 1975), cultured mouse spinal neurones (Barker & Ransom, 1978) and sympathetic ganglia (Brown & Constanti, 1978). Several experiments have shown that the presynaptic release of GABA may be inhibited by barbiturates (Cutler, Markowitz & Dudzinski, 1974; Haycock, Levy & Cotman, 1977; Jessel & Richards, 1977).

Excitatory synaptic transmission is apparently depressed by many anaesthetics and this may be due to decreased release of excitatory substances such as L-aspartate or L-glutamate, enhancement of their uptake or depression of their postsynaptic effects. Evidence has been adduced that barbiturates depress the sensitivity of neurones in the cerebral cortex and cuneate nucleus to excitatory substances (Galindo, 1969; Crawford, 1970) and in the olfactory cortex *in vitro* the sensitivity of neurones to electrophoretically applied glutamate was similarly reduced by pentobarbitone (Richards & Smaje, 1976). Furthermore, using intracellular recording techniques Weakly (1969) showed that barbiturates depressed the quantal release of an (unknown) excitatory transmitter on spinal neurones.

The present experiments were designed to investigate the effects of a variety of intravenous anaesthetics upon the release of a depressant (GABA) and an excitant (aspartate) amino acid and on their uptake mechanisms. A preliminary account of some of the results has been given (Minchin, 1980a).

[1] Present address: MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE.

0007-1188/81/070681-09 $01.00

## Methods

### *Release experiments*

Adult albino rats (Sheffield strain) were decapitated and their brains quickly removed on to an ice-cold surface. The cerebral cortices were dissected, the pia were removed and the tissue was chopped in two directions on a McIlwain tissue chopper to give slices of dimension 0.1 × 0.1 × 1.5 mm. These were suspended in Krebs-phosphate solution (composition mM: NaCl 118, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4$ 1.2, D-glucose 5.6 and sodium phosphate buffer (pH 7.4) 15) and portions containing 50 mg tissue were distributed into flasks so that the final volume was 5 ml. The flasks were placed in a shaking water bath at 37°C for 10 min after which D-[2,3-$^3$H] aspartic acid (Radiochemical Centre, Amersham, sp. act. 18 Ci/mmol) and 4-amino-n-[U-$^{14}$C]-butyric acid (Radiochemical Centre, Amersham, sp. act. 226 mCi/mmol) were added to give final concentrations of 6.7 nM and 0.3 μM, respectively. Incubation was continued for 15 min and the slices were collected by filtration on to Whatman GF/A filters, rinsed and mounted in Sartorius membrane filter holders (Sartorius G.m.b.H., Göttingen, Germany). They were then superfused with warm, oxygenated Krebs-phosphate solution (0.5 ml/min). All the solutions in these experiments contained amino oxyacetic acid (10 μM) to inhibit metabolism of GABA by 4-aminobutyrate-2-oxoglutarate aminotransferase (EC.2.6.1.19). Metabolism of aspartate was also avoided since although D-aspartate is taken up by the same carrier that takes up L-aspartate (and probably also L-glutamate) it is not metabolized by brain tissue (Davies & Johnston, 1976). Fractions of superfusate were collected every 3 min and the radioactivity therein was estimated by liquid scintillation spectrometry after the addition of 10 ml of 0.3% diphenyloxazole in a mixture of toluene:Triton X-100 (2:1 v/v). At the end of the experiment, the radioactivity remaining in the tissue was extracted into 1.3 ml water and similarly counted. Efficiencies were calculated by the external standard ratio method and correction made for $^3$H spillover into the $^{14}$C channel and *vice versa*. The radioactivity of each isotope in each wash was expressed as an approximate first order efflux rate constant, which was calculated as $\ln(D_o/D_t)/t$, where $D_o$ = d/min in tissue at the start of the wash period, $D_t$ = d/min in tissue at the end of the wash period and t = duration of the wash period. Collection of fractions was routinely started 20 min after the start of superfusion to allow for the washout of extracellular radioactivity and to enable the efflux of the amino acids to reach a steady value.

### *Uptake experiments*

Portions of the tissue suspension containing 10 mg of slices were added to flasks containing Krebs-phosphate solution with amino oxyacetic acid (10 μM) so that the final volume was 10 ml. The flasks were preincubated at 37°C for 10 min and D-[$^3$H]-aspartic acid and [$^{14}$C]-GABA were added to give final concentrations of 0.28 nM and 11 nM, respectively. After a further incubation of 10 min the slices were collected on to Whatman No.1 filters, rinsed with 5 ml ice-cold Krebs-phosphate solution and their radioactivity measured after extraction into 1.3 ml water and addition of 10 ml scintillation mixture. When drugs were present they were added before the preincubation period. Blanks were run in which both preincubation and incubation with radioactive substances took place at 0°C.

### *Materials*

Protoveratrine A, amino oxyacetic acid hemihydrochloride and Cremophor EL were obtained from Sigma. Ketamine hydrochloride was a gift from Parke, Davis & Co., Pontypool, Gwent, α-chloralose was prepared by filtering a hot solution of chloralose and allowing the filtrate to cool, when α-chloralose precipitated. The precipitate was recrystallised from ethanol twice. Urethane (ethyl carbamate), thiopentone (Intraval), pentobarbitone, methohexitone (Brietal Sodium), hydroxydione (Viadril) and alphaxalone/alphadolone (Saffan) were obtained from commercial suppliers.

It is possible that some of the hydroxydione used in the experiments had hydrolysed to release succinate and the parent steroid since the sample was several years old, although it was stored dry and in the dark. However, it is believed that hydrolysis occurs *in vivo* and that the active substance is, in fact, the parent steroid. The sample of Viadril used in this study was tested and found to be anaesthetic in a rat when given intravenously at a dose of 60 mg/kg.

## Results

### *Effect of $Ca^{2+}$ on release*

Exposure of the slices to 40 mM $K^+$ (as KCl replacing NaCl in the Krebs-phosphate solution) enhanced the efflux of both GABA and D-aspartate, the former to a greater extent than the latter. This was greatly diminished in the case of GABA if $Ca^{2+}$ was omitted from the superfusing medium for 6 min before and during exposure to elevated $K^+$ concentration (Figure 1a). The $K^+$-evoked release of D-aspartate was

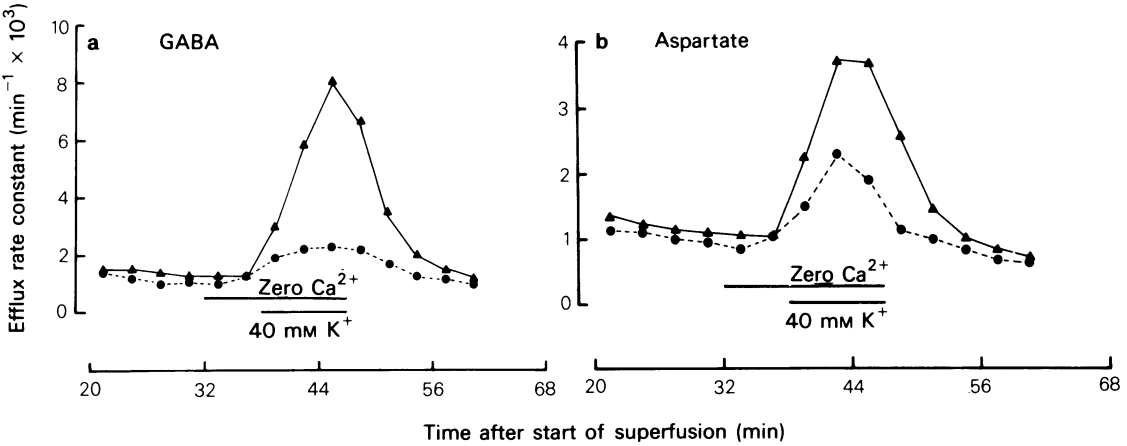


**Figure 1** The effect of removing $Ca^{2+}$ from the superfusing medium on the $K^+$-induced release of (a) [$^3$H]-γ-aminobutyric acid ([$^3$H]-GABA) and (b) [$^3$H]-D-aspartate. Slices of cerebral cortex were incubated with [$^3$H]-GABA or [$^3$H]-D-aspartate and superfused as described in Methods. (▲) Control slices were depolarized with 40 mM $K^+$ as shown by the short horizontal line; (●) experimental slices were superfused with $Ca^{2+}$-free medium for 6 min before and during exposure to 40 mM $K^+$ as shown by the long horizontal line. Each point is the mean of 3 experiments.

also considerably reduced by this procedure, though to a lesser extent than that of GABA (Figure 1b).

*The effect of barbiturates on release*

In control experiments the maximum percentage increase in the efflux rate constant of GABA caused by a 9 min exposure to 40 mM $K^+$ was 479 ± 16% (s.e.mean, $n = 160$). The corresponding figure for D-aspartate was 223 ± 6% ($n = 183$). In some experiments 10 μM protoveratrine A was used as the depolarizing agent, in this case a 9 min exposure caused an increase of 275 ± 40% ($n = 18$) in the efflux rate constant for GABA.

Thiopentone, at concentrations above 10 μM, inhibited the $K^+$-stimulated release of GABA; below 10 μM inconsistent effects were observed, none of which were significant (Figure 2a). A similar influence on D-aspartate release was seen, except that it was slightly less sensitive to the effects of thiopentone (Figure 2a). In addition, protoveratrine-induced release of GABA was inhibited by thiopentone, though consistent inhibition was only seen at concentrations above 50 μM (Figure 2a). Protroveratrine is a depolarizing agent that is thought to act on excitable tissues only and does not appear to induce release from glial cells (Minchin, 1980b). Pentobarbitone had a qualitatively similar effect on GABA and D-aspartate release, though it was less potent (Figure 2b). Similarly, methohexitone inhibited the $K^+$-stimulated release of both amino acids and it too was less potent than thiopentone (Figure 2c): None of the barbiturates showed any enhancement of efflux at low concentrations.

*Steroid anaesthetics*

Hydroxydione, a water-soluble steroid, inhibited the $K^+$-stimulated release of both GABA and D-aspartate at concentrations above 40 μM (Figure 2d). As with the barbiturates, GABA efflux was more sensitive to the influence of the anaesthetic than D-aspartate. Alphaxalone/alphadolone by contrast had no significant effect upon the spontaneous or $K^+$-stimulated efflux of GABA and D-aspartate at concentrations between 1 and 300 μM although there was a small but insignificant inhibition at the highest concentration. In these experiments the controls were run with the appropriate concentration of Cremophor EL, the solvent for the steroid mixture, although this had no discernible effect upon the efflux of either amino acid.

*Ketamine, urethane and α-chloralose*

Ketamine (1–100 μM), urethane (0.5–30 mM) or α-chloralose (30–1000 μM) did not influence spontaneous or $K^+$-evoked release of either GABA or D-aspartate. In the case of 30 mM urethane, controls were run with 30 mM sucrose to allow for any influence of osmotic pressure, although none was evident.

*Effect of barbiturates on release in the absence of $Ca^{2+}$*

To determine whether the anaesthetic-induced in-

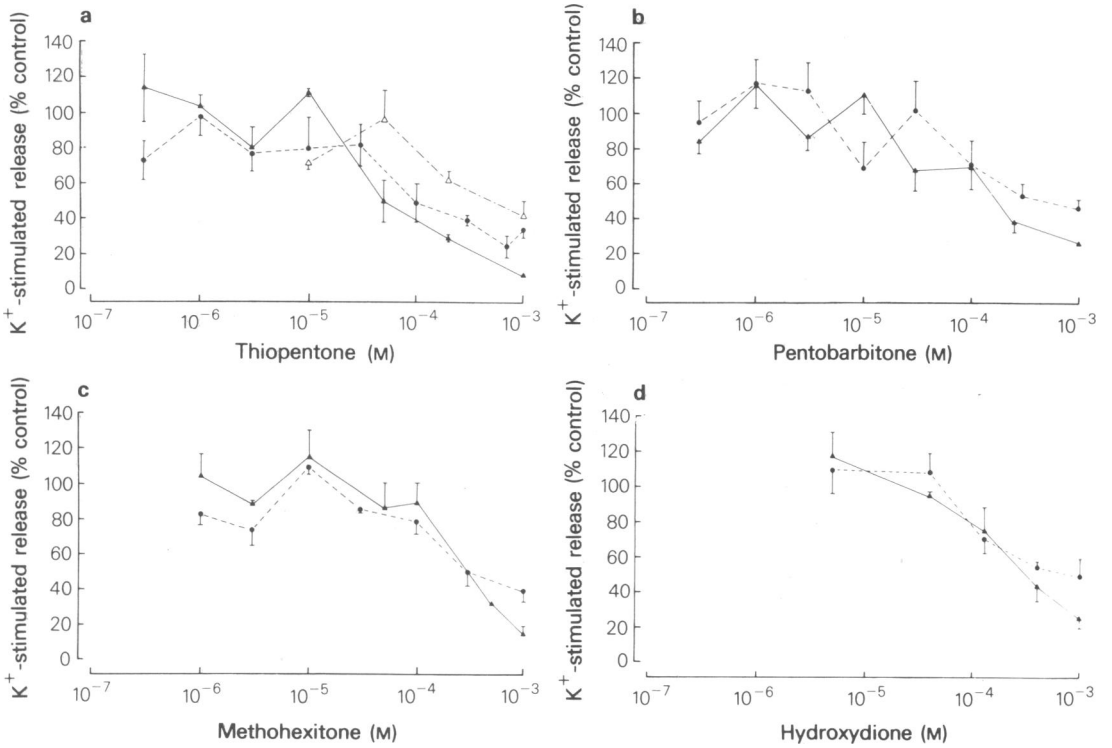


**Figure 2** Effect of (a) thiopentone, (b) pentobarbitone, (c) methohexitone and (d) hydroxydione on the $K^+$-stimulated release of [$^{14}$C]-γ-aminobutyric acid ([$^{14}$C]-GABA) and [$^3$H]-D-aspartate. Slices were incubated with [$^{14}$C]-GABA and [$^3$H]-D-aspartate and then superfused as described in Methods. Thirty-eight min after the start of superfusion control slices were depolarized with 40 mM $K^+$ for 9 min. Experimental slices were exposed to various concentrations of anaesthetic for 6 min before and during depolarization and the increase in release due to the elevated $K^+$ concentration was expressed as a percentage of the increase seen in controls that were run in parallel. None of the anaesthetics altered spontaneous efflux of either [$^{14}$C]-GABA or [$^3$H]-D-aspartate. (▲) GABA release; (●---●) D-aspartate release; (△) GABA release induced by 10 μM protoveratrine A. Each point is the mean of 3–8 experiments with s.e.mean indicated by vertical bars.

hibition of release was due to an action solely on nerve terminals, experiments were repeated in the absence of $Ca^{2+}$, a condition known to abolish release from nerve terminals. None of the barbiturates modified the spontaneous efflux of GABA and D-aspartate when the slices were superfused with Krebs-phosphate solution in which $Ca^{2+}$ had been removed and the $Mg^{2+}$ concentration raised to 10 mM. However varying effects were seen upon the $K^+$-stimulated release of the amino acids, which in the controls was much reduced. Thiopentone, at the concentration which gave maximum inhibition of release in normal Krebs-phosphate did not significantly alter the release of GABA in the low $Ca^{2+}$, high $Mg^{2+}$ medium although D-aspartate release was increased somewhat (Figure 3). However, pentobarbitone at the concentration of maximum effectiveness in normal medium inhibited $K^+$-stimulated GABA release, albeit by only 40%, whilst the release of D-aspartate was enhanced, though not significantly (Figure 3). Finally, methohexitone at the maximum inhibitory concentration in normal medium inhibited the release of both GABA and D-aspartate by about 70% in the low $Ca^{2+}$, high $Mg^{2+}$ medium (Figure 3).

*Effect of anaesthetics on transmitter uptake*

Thiopentone, methohexitone, ketamine, urethane and α-chloralose all failed to alter significantly the uptake of GABA and D-aspartate at any of the concentrations tested (Table 1). However, pentobarbitone weakly inhibited uptake of both amino acids, inhibition appearing to reach a maximum of approximately 20% at 100 μM pentobarbitone (Table 1). Hydroxydione was a moderately potent inhibitor of

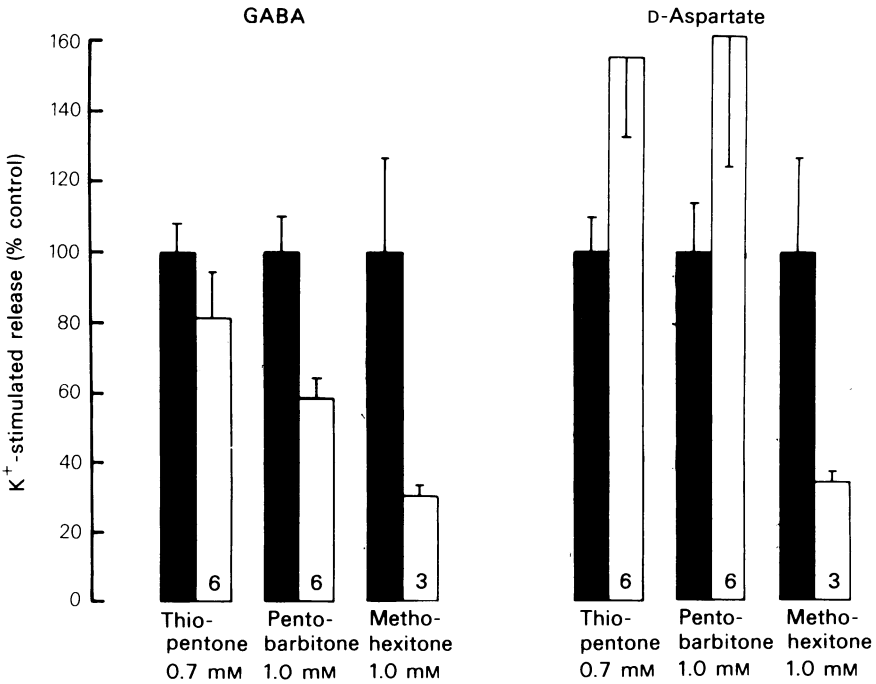


**Figure 3** The effect of low $Ca^{2+}$ medium on the inhibition of $K^+$-stimulated release of [$^{14}$C]-γ-aminobutyric acid ([$^{14}$C]-GABA) and [$^3$H]-D-aspartate by barbiturates. Slices were incubated and superfused as described in Methods, except that the superfusing medium lacked $Ca^{2+}$ and contained 10 mM $Mg^{2+}$. Control slices were depolarized with 40 mM $K^+$ for 9 min after 38 min superfusion with $Ca^{2+}$-free medium, whilst experimental samples were exposed to barbiturates for 6 min before and during depolarization. None of the barbiturates altered the spontaneous efflux of either amino acid. The increase in release caused by 40 mM $K^+$ in the experimental slices is expressed as a percentage of that seen in controls. Filled columns represent normalized control values and open columns show release in the presence of barbiturate. Vertical lines indicate s.e.mean and the number of experiments is shown in the open columns.

the uptake of D-aspartate and less potent against GABA; $IC_{50}$ values were calculated from log-probability plots, which gave values of 900 μM for GABA and 600 μM for D-aspartate. Alphaxalone/alphadolone slightly inhibited uptake of both amino acids at 1 μM but was considerably more effective at 300 μM (Table 1). In these experiments, control uptake was measured in the presence of the appropriate concentration of Cremophor EL, although uptake in the presence of this solvent was not significantly different from uptake in its absence at any of the concentrations.

## Discussion

Calculated anaesthetic concentrations in rodents for the anaesthetics used in this study are shown in Table 2. The figures may be considered to approximate the concentration of free drug during the steady state phase of anaesthesia but higher and lower concentrations would be found during induction and recovery. The likely range of anaesthetic concentrations has therefore been covered for the drugs used in the present investigation.

There were no consistent effects of the anaesthetics on amino acid uptake. Of the barbiturates, only pentobarbitone showed inhibitory activity which was very weak. This is similar to the lack of effect of pentobarbitone seen at concentrations up to 1 mM on GABA uptake into slices of hippocampus (Jessell & Richards, 1977) and up to 750 μM in olfactory cortex slices (Collins, 1980). It would appear that where pentobarbitone has been shown to influence amino acid uptake its effects have been weak; it is therefore unlikely that they contribute significantly to the general anaesthetic effects of this barbiturate.

The inhibition of uptake shown by hydroxydione was too weak to occur to any great extent at anaesthetic concentrations. Similarly, the effect of alphaxalone/alphadolone was weak at anaesthetic concentrations. The bizarre relationship between the

**Table 1** Effect of anaesthetics on uptake of D-[$^3$H]-aspartate and [$^{14}$C]-γ-aminobutyric acid [$^{14}$C]-GABA)

| Drug | Concentration (μM) | GABA uptake (% of control) | P | D-Aspartate uptake (% of control) | P |
|---|---|---|---|---|---|
| Thiopentone | 10 | 102 ± 10 (4) | NS | 100 ± 11 (4) | NS |
| | 100 | 115 ± 13 (8) | NS | 113 ± 13 (8) | NS |
| | 700 | 101 ± 5 (4) | NS | 92 ± 3 (4) | NS |
| Pentobarbitone | 10 | 107 ± 7 (4) | NS | 110 ± 8 (4) | NS |
| | 100 | 83 ± 5 (7) | <0.025 | 84 ± 5 (7) | <0.025 |
| | 1000 | 82 ± 5 (8) | <0.05 | 78 ± 5 (8) | <0.01 |
| Methohexitone | 1 | 83 ± 5 (3) | NS | 82 ± 6 (3) | NS |
| | 10 | 77 ± 8 (7) | NS | 78 ± 9 (7) | NS |
| | 100 | 123 ± 15 (4) | NS | 122 ± 16 (4) | NS |
| | 1000 | 101 ± 14 (7) | NS | 86 ± 13 (7) | NS |
| Hydroxydione | 10 | 114 ± 7 (4) | NS | 112 ± 7 (4) | NS |
| | 100 | 85 ± 7 (8) | NS | 78 ± 6 (8) | <0.01 |
| | 200 | 70 ± 6 (4) | NS | 64 ± 5 (4) | <0.05 |
| | 400 | 65 ± 5 (5) | <0.001 | 57 ± 5 (5) | <0.001 |
| Alphaxalone/ Alphadolone | 1 | 81 ± 5 (7) | <0.005 | 80 ± 6 (7) | <0.005 |
| | 10 | 111 ± 9 (8) | NS | 115 ± 9 (8) | NS |
| | 100 | 110 ± 9 (4) | NS | 114 ± 9 (4) | NS |
| | 300 | 66 ± 7 (4) | <0.02 | 63 ± 8 (4) | <0.02 |
| Ketamine | 100 | 97 ± 8 (8) | NS | 97 ± 9 (8) | NS |
| Urethane | 15000 | 87 ± 7 (4) | NS | 88 ± 8 (4) | NS |
| α-Chloralose | 200 | 113 ± 10 (4) | NS | 111 ± 9 (4) | NS |

Results are the mean ± s.e.mean with the number of experiments in parentheses. Statistical test was the 2-tailed *t* test. NS = not significant.

concentration of this steroid mixture and inhibition of amino acid uptake suggests an interaction other than competitive inhibition.

The $K^+$-stimulated release of both amino acids was largely $Ca^{2+}$-dependent. Since most neurohumoral release processes are known to require extracellular $Ca^{2+}$, it is likely that the released GABA, and D-aspartate originated from nerve terminals. However, exogenous amino acids may be taken up by glial cells and nerves under some circumstances (Wheeler & Boyarski, 1968; Schon & Kelly, 1973; Bowery, Brown, White & Yamini, 1979) and can be released from glial cells by elevated extracellular $K^+$ concentrations (Bowery & Brown, 1972; Minchin & Iversen, 1974). However, there is some uncertainty as to whether elevated $K^+$ concentration releases amino acids from nerves (De Feudis, 1971; Weinreich & Hammerschlag, 1975). Furthermore, while $K^+$-stimulated release from peripheral glia is at least partially $Ca^{2+}$-dependent (Minchin & Iversen, 1974; Roberts, 1974), that from bulk isolated central glia appears not to be (Sellström & Hamberger, 1977). It is possible, therefore, that the $Ca^{2+}$-independent release of GABA and D-aspartate originated from glia and/or nerve fibres.

The inhibition of $K^+$-stimulated amino acid efflux by barbiturates seen in this study confirms several previous observations (Cutler *et al.*, 1974; Haycock *et al.*, 1977; Jessell & Richards, 1977; Cutler & Young, 1979; Willow, Bornstein & Johnston, 1980). In the case of thiopentone, the inhibition of release of both GABA and D-aspartate appears to be a neuronal phenomenon since release in low $Ca^{2+}$, high $Mg^{2+}$ media was not inhibited, whilst that induced by protoveratrine was. However, pentobarbitone inhibited GABA release from non-neuronal elements, though the contribution of this to the inhibition seen in normal media would be small since $K^+$-stimulated release of GABA from non-neuronal sources is only a small proportion of the total. By contrast, pentobarbitone-induced inhibition of D-aspartate release seems to occur exclusively at neuronal sites. In the case of methohexitone a different pattern was seen, with large inhibitions of both GABA and D-aspartate release from non-neuronal sources. Clearly the structural specificity of barbiturate effects on amino acid release processes in non-neuronal elements is different from that in nerve terminals, although in normal media effects on nerve terminals are predominant.

The mechanism of the inhibition of amino acid release seen with barbiturates deserves some consideration. It has been known for some time that barbiturates enhance GABA-mediated inhibitions and

**Table 2** Anaesthetic doses and approximate concentrations in rodents

| *Anaesthetic* | *Anaesthetic dose* (mg/kg) | *Anaesthetic conc.* (μM) | *References* |
|---|---|---|---|
| Thiopentone | 13.2 (i.v.) | 50 | Mouse, $AD_{50}$: Child, Currie, Davis, Dodds, Pearce & Twissell, 1971. |
| Pentobarbitone | 25.0 (i.v.) | 92 | Rat: Barnes & Eltherington, 1973. |
| Methohexitone | 5.4 (i.v.) | 19 | Mouse, $AD_{50}$: Child *et al.*, 1971. |
| Hydroxydione | 18.0 (i.v.) | 60 | Mouse, $AD_{50}$: Child *et al.*, 1971. |
| Alphaxalone/ alphadolone | 1.8 (i.v.) | 11 | Mouse, $AD_{50}$: Child *et al.*, 1971. |
| Urethane | 780.0 (i.p.) | 13,279 | Rat: Barnes & Eltherington, 1973. |
| α-Chloralose | 55.0 (i.p.) | 270 | Rat: Barnes & Eltherington, 1973. |
| Ketamine | 12.7 (i.v.) | 70 | Mouse, $AD_{50}$: Child *et al.*, 1971. |

Approximate anaesthetic concentrations were calculated for fast-acting anaesthetics (thiopentone, methohexitone, alphaxalone/alphadolone) assuming that they distribute throughout the extracellular water (approx. 30% of body weight) during anaesthesia (Richards, 1972) and for slower acting substances assuming that they distribute evenly throughout the total body water (approx. 66% of body weight (Altman & Dittmer, 1974)). In addition, correction was made for protein binding of some anaesthetics as follows: 70% of thiopentone and methohexitone assumed bound (Davis, 1975), 40% of alphaxalone/alphadolone assumed bound (Child, Gibson, Harnby & Hart, 1972) and 40% of pentobarbitone assumed bound (Dundee, 1974). $AD_{50}$ = anaesthetic $dose_{50}$.

recently it has become clear that while barbiturates do not bind to the GABA receptor itself they do appear to interact with the chloride ionophore associated with the GABA receptor (Ticku & Olsen, 1978). GABA release has been shown to be influenced by GABA receptor agonists and antagonists (Johnston & Mitchell, 1971; Mitchell & Martin, 1978; Snodgrass, 1978), suggesting feedback inhibition of GABA release by presynaptic GABA receptors. It is possible, therefore, that if a chloride ionophore is associated with this receptor, in a fashion analagous to that at postsynaptic receptors, barbiturates may act as agonists at this site to inhibit GABA efflux.

Under some conditions barbiturates have been shown to enhance GABA efflux from various neuronal preparations. For example, 100 μM pentobarbitone enhanced the release of endogenous GABA from olfactory cortex slices induced by stimulation of the lateral olfactory tract (Collins, 1980), and at 1 mM this effect reversed to one of inhibition. Similarly, the electrically stimulated release of newly synthesized GABA from guinea-pig cortex slices was also enhanced by pentobarbitone (100 μM) (Potashner, Lake, Langlois, Plouffe & Lecavalier, 1980). Finally, in rat thalamic slices the $K^+$-stimulated release of [$^3$H]-GABA was enhanced at low barbiturate concentrations and inhibited at high concentrations (Kendall, Minchin & Angel, unpublished observation). Therefore the effect of barbiturates on amino acid release depends not only upon concentration, but also upon differences in regional susceptibility.

The rank order of potency of methohexitone, thiopentone and pentobarbitone as anaesthetics did not correlate with their potency as inhibitors of the release of either GABA or D-aspartate. Furthermore, although thiopentone and pentobarbitone inhibited transmitter release by 40–50% at concentrations that have been shown to affect synaptic transmission (Richards, 1972) and are close to those expected *in vivo* during anaesthesia, inhibition of release by methohexitone and hydroxydione was very weak at these concentrations, and ketamine, chloralose, alphaxalone/alphadolone and urethane were ineffective at any of the concentrations tested. It is not clear why alphaxalone/alphadolone, which are structurally (but not conformationally) similar to hydroxydione, failed to influence release; higher concentrations may be necessary to demonstrate this effect. However, it is clear that whilst inhibition of excitatory transmitter release may be one of a number of causes of anaesthesia for some substances (though the parallel inhibition of inhibitory transmitter release might be expected to antagonize such an effect) for many of the drugs tested in the present study no such mechanism appears to be involved.

## References

ALTMAN, P.L. & DITTMER, D.S. (1974). Blood and other body fluids. In *Biology Data Book*, Vol. III, ed. Altman, P.L. & Dittmer, D.S., p 1991. Bethesda: Federation of American Societies for Experimental Biology.

BARKER, J.L., RANSOM, B.R. (1978). Pentobarbitone pharmacology of mammalian central neurones grown in tissue culture. *J. Physiol.*, **280,** 355–372.

BARNES, C.D. & ELTHERINGTON, L.G. (1973). *Drug Dosage in Laboratory Animals.* Berkeley & Los Angeles, California, U.S.A: University of California Press.

BOWERY, N.G. & BROWN, D.A. (1972). γ-Aminobutyric acid uptake by sympathetic ganglia. *Nature, New Biol.*, **238,** 89–91.

BOWERY, N.G., BROWN, D.A., WHITE, R.D. & YAMINI, G. (1979). [$^3$H]γ-aminobutyric acid uptake into neuroglial cells of rat superior cervical sympathetic ganglia. *J. Physiol.*, **293,** 51–74.

BROWN, D.A. & CONSTANTI, A. (1978). Interaction of pentobarbitone and γ-aminobutyric acid on mammalian sympathetic ganglion cells. *Br. J. Pharmac.*, **63,** 217–224.

CHILD, K.J., CURRIE, J.P., DAVIS, B., DODDS, M.G., PEARCE, D.R. & TWISSEL, A.B. (1971). The pharmacological properties in animals of CT 1341 – a new steroid anaesthetic agent. *Br. J. Anaesth.*, **43,** 2–13.

CHILD, K.J., GIBSON, W., HARNBY, G. & HART, J.W. (1972). Metabolism and excretion of Althesin (CT 1341) in the rat. *Postgrad. med. J. (Supple. 2),* **48,** 37–43.

COLLINS, G.G.S. (1980). Release of endogenous amino acid neuro-transmitter candidates from rat olfactory cortex slices: possible regulatory mechanisms and the effects of pentobarbitone. *Brain Res.*, **190,** 517–528.

CRAWFORD, J.M. (1970). Anaesthetic agents and the chemical sensitivity of cortical neurones. *Neuropharmacology,* **9,** 31–46.

CUTLER, R.W.P., MARKOWITZ, D. & DUDZINSKI, D.S. (1974). The effect of barbiturates on [$^3$H]GABA transport in rat cerebral cortex slices. *Brain Res.*, **81,** 189–197.

CUTLER, R.W.P. & YOUNG, J. (1979). Effect of barbiturates on release of endogenous amino acids from rat cortex slices. *Neurochem. Res.*, **4,** 319–329.

DAVIES, L.P. & JOHNSTON, G.A.R. (1976). Uptake and release of D- and L-aspartate by rat brain slices. *J. Neurochem.*, **26,** 1007–1014.

DAVIS, B. (1975). Noninhalational anaesthetics. *Adv. Drug Res.*, **10,** 1–51.

DE FEUDIS. F.V. (1971). Effects of electrical stimulation on the efflux of L-glutamate from peripheral nerve *in vitro. Exp. Neurol.*, **30,** 291–296.

DUNDEE, J.W. (1974). Molecular structure-activity relationships of barbiturates. In *Molecular Mechanisms in General Anaesthesia.* ed. Halsey, M.J., Millar, R.A. & Sutton, J.A. pp. 16–30. Edinburgh: Churchill Livingstone.

ECCLES, J.C., FABER, D.S. & TÁBOŘÍKOVÁ, H. (1971). The action of a parallel fiber volley on the antidromic invasion of Purkinje cells of cat cerebellum. *Brain Res.*, **25,** 335–356.

ECCLES, J.C., SCHMIDT, R. & WILLIS, W.D. (1963). Pharmacological studies on presynaptic inhibition. *J. Physiol.*, **168,** 500–530.

GALINDO, A. (1969). Effects of procaine, pentobarbital and halothane on synaptic transmission in the central nervous system. *J. Pharmac. exp. Ther.*, **169,** 185–195.

HAYCOCK, J.W., LEVY, W.B. & COTMAN, C.W. (1977). Pentobarbital depression of stimulus-secretion coupling in brain-selective inhibition of depolarization-induced calcium-dependent release. *Biochem. Pharmac.*, **26,** 159–161.

JESSELL, T.M. & RICHARDS, C.D. (1977). Barbiturate potentiation of hippocampal i.p.s.p.s. is not mediated by blockade of GABA uptake *J. Physiol.*, **269,** 42–44P.

JOHNSTON, G.A.R. & MITCHELL, J.F. (1971). The effect of bicuculline, metrazol, picrotoxin and strychnine on the release of $^3$H-GABA from rat brain slices. *J. Neurochem.*, **18,** 2441–2446.

MINCHIN, M.C.W. (1980a). The effect of some anaesthetic agents on [$^3$H]-GABA release from rat brain slices. *Br. J. Pharmac.*, **68,** 131P.

MINCHIN, M.C.W. (1980b). Veratrum alkaloids as transmitter-releasing agents. *J. Neurosci. Methods,* **2,** 111–121.

MINCHIN, M.C.W. & IVERSEN, L.L. (1974). Release of [$^3$H] gamma-aminobutyric acid from glial cells in rat dorsal root ganglia. *J. Neurochem.*, **23,** 533–540.

MITCHELL, P.R. & MARTIN, I.L. (1978). Is GABA release modulated by presynaptic receptors? *Nature,* **274,** 904–905.

NICOLL, R.A. (1972). The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. *J. Physiol.*, **223,** 803–814.

NICOLL, R.A. (1975). Pentobarbital: action on frog motoneurons. *Brain Res.*, **96,** 119–123.

NICOLL, R.A., ECCLES, J.C., OSHIMA, T. & RUBIA, F. (1975). Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. *Nature,* **258,** 625–627.

POTASHNER, S.J., LAKE, N., LANGLOIS, E.A., PLOUFFE, L. & LECAVALIER, D. (1980). Pentobarbital: differential effects on amino acid transmitter release. In *Molecular Mechanisms of Anaesthesia* (Progress in Anaesthesiology, Vol. 2), ed. Fink, B.R. pp. 469–476. New York: Raven Press.

RICHARDS, C.D. (1972). On the mechanism of barbiturate anaesthesia. *J. Physiol.*, **227,** 749–767.

RICHARDS, C.D. & SMAJE, J.C. (1976). Anaesthetics depress the sensitivity of cortical neurones to L-glutamate. *Br. J. Pharmac.*, **58,** 347–357.

ROBERTS, P.J. (1974). Amino acid release from isolated dorsal root ganglia. *Brain Res.*, **74,** 327–332.

SCHON, F. & KELLY, J.S. (1973). Autoradiographic localisation of [$^3$H]GABA and [$^3$H]glutamate over satellite glial cells. *Brain Res.*, **66,** 275–288.

SELLSTRÖM, Å. & HAMBERGER, A. (1977). Potassium-stimulated γ-aminobutyric acid release from neurons and glia. *Brain Res.*, **119,** 189–198.

SNODGRASS, S.R. (1978). Use of $^3$H-muscimol for GABA receptor studies. *Nature,* **273,** 392–394.

TICKU, M.K. & OLSEN, R.W. (1978). Interaction of barbiturates with dihydropicrotoxinin binding sites related to the GABA receptor-ionophore system. *Life Sci.*, **22,** 1643–1652.

WEAKLY, J.N. (1969). Effect of barbiturates on 'quantal' synaptic transmission in spinal motoneurones. *J. Physiol.*, **204,** 63–77.

WEINREICH, D. & HAMMERSCHLAG, R. (1975). Nerve

impulse-enhanced release of amino acids from non-synaptic regions of peripheral and central nerve trunks of bullfrog. *Brain Res.*, **84,** 137–142.

WHEELER, D.D. & BOYARSKY, L.L. (1968). Influx of glutamic acid in peripheral nerve-characteristics of influx. *J. Neurochem.*, **15,** 1019–1031.

WILLOW, M., BORNSTEIN, J.C. & JOHNSTON, G.A.R. (1980). The effects of anaesthetic and convulsant barbiturates on the efflux of [$^3$H]D-aspartate from brain minislices. *Neurosci. Lett.*, **18,** 185–190.

*(Received November 26, 1980.*
*Revised March 13, 1981.)*